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Pollen morphology and its taxonomic value in *Brachylaena* (Asteraceae) in southern Africa

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The pollen morphology of all the southern African representatives of *Brachylaena* R. Br. was investigated using light and scanning electron microscopy. On the basis of pollen morphology it is possible to recognize two groups in the genus. *B. discolor* (including *B. uniflora*), *B. elliptica*, *B. glabra*, *B. neriifolia* and *B. rotundata* are included in Group I, while *B. huillensis* and *B. ilicifolia* are the only representatives of Group II. The differences between the groups primarily concern length and width of colpi, shape of colpus ends, length and width of spinulae, frequency of spinulae, finer tectum sculpture and exine stratification. The possible taxonomic significance of pollen morphology is briefly discussed.

Die stuifmeelmorfologie van al die suider-Afrikaanse verteenwoordigers van *Brachylaena* R. Br. is met behulp van lig- en aftas-elektronmikroskopie ondersoek. Op grond van stuifmeelmorfologie kan twee groepe binne die genus onderskei word. *B. discolor* (insluitend *B. uniflora*), *B. elliptica*, *B. glabra*, *B. neriifolia* en *B. rotundata* ressorteer in Groep I, terwyl *B. huillensis* en *B. ilicifolia* die enigste verteenwoordigers van Groep II is. Die belangrikste verskille tussen die twee groepe is ten opsigte van lengte en breedte van kolpusse, vorm van die kolpuspunte, lengte en breedte van spinulas, frekwensie van spinulas, fyner skulptuur van die tektum en eksienstratifikasie. Die moontlike taksonomiese waarde van stuifmeelmorfologie word kortliks bespreek.

Keywords: Asteraceae, *Brachylaena*, pollen morphology.

Introduction

Thorough palynological studies on certain representatives of the Asteraceae have been carried out by various authors [Wodehouse (1935) on external morphology and Stix (1960) on internal morphology]. These studies usually concentrated on taxonomically useful characters. Since the usefulness of pollen as a taxonomic tool is restricted by the number of characters that can be observed by means of light microscopy (LM) (Wodehouse, 1928), it became apparent to taxonomists that additional pollen characters would have to be obtained by using transmission (TEM) and scanning (SEM) electron microscopes (Skvarla *et al.*, 1977).

As early as 1966 Skvarla and Turner reported on TEM studies of pollen of representatives of most of the tribes of the Asteraceae, while Clark *et al.* (1980), Tomb *et al.* (1974) and others did SEM studies on a selection of representatives of the family. In spite of the palynological research in the family, relatively few studies on comparative pollen morphology of taxa were carried out at infrageneric level (Keeley & Jones, 1977).

To date, pollen of *Brachylaena* R. Br. has been studied by means of LM only (Erdtman 1966; Leins 1971). The pollen of *Brachylaena* is tricolporate and subprolate with an exine stratification similar to that of *Tarchonanthus* (Erdtman, 1966). Material from one locality only of each of *Brachylaena discolor*, *B. rotundata* and *B. neriifolia* were studied by Erdtman (1966).

Based on LO analyses of the pollen of most of the members of the tribe Inuleae, Leins (1971) distinguished three pollen types in the subtribe Tarchonantheae (to which *Brachylaena* belongs), mainly on the basis of exine stratification. The *Tarchonanthus camphoratus* pollen type was found by Leins (1971) in four *Brachylaena* species (*B. discolor*, *B. rotundata*, *B. neriifolia* and *B. elliptica*) and four *Tarchonanthus* species. The sexine of the pollen of this type is 1.6 μm thick and consists of two layers of baculae (Leins, 1971). The nexine is 0.8 – 1 μm thick and consists of two parts (nexine 1 and 2). He also mentioned that these pollen grains are tricolporate and possess narrow colpi with pointed ends, while the tectum is covered with spinulae. The *B. huillensis* pollen type was found in *B. huillensis* and *B. ilicifolia* only and the sexine of their grains is 0.7 μm thick and consists of a single bacula layer. The nexine is undifferentiated and is 0.5 μm thick. These pollen grains are tri- or tetracolporate and possess much wider colpi with rounded ends and the tectum is also covered by spinulae. The *Brachylaena ramiflora* pollen type is unique to this Madagascar species.

Leins (1971) also questioned the validity of the inclusion of the subtribe Tarchonantheae in the tribe Inuleae, on the basis of their pollen morphology. He recommended the classification of this subtribe in the tribe Mutisieae, while Skvarla *et al.* (1977) suggested its inclusion within the tribe Anthemidae, also based on pollen morphology. *Brachylaena* was also placed in three other tribes over the years. Keeley and Jansen (1991), however, recognized a new tribe, the Tarchonantheae, with *Brachylaena* and *Tarchonanthus* the only members, on the basis of chloroplast DNA variation.

Unfortunately, Leins (1971) did not study pollen of all the southern African species of the genus. He also ignored some representatives of the taxonomically problematic *B. discolor–uniflora* species complex and in five cases he based his results on pollen from only one specimen.

To determine whether pollen morphological characters have taxonomic value amongst the southern African representatives of *Brachylaena*, it is essential to gain information from specimens of all the taxa collected from various localities. The aim of this study was to search for additional taxonomically useful characters by using the SEM. To check whether the results of Leins (1971) on exine stratification hold true for all the *Brachylaena* taxa in southern Africa, the SEM studies were complemented with LM studies.

Materials and Methods

Pollen of all the southern African taxa of *Brachylaena* [*B. uniflora* is included in *B. discolor* subsp. *transvaalensis*, (Cilliers, 1990)] were studied by means of LM and SEM. Pollen from herbarium specimens from a minimum of four localities each were studied (Table 1).

Data on the following characters were recorded:

(a) Polarity, size and symmetry of pollen grains (SEM and LM).

(b) Shape, as well as the amb: equatorial diameter (SEM and LM); polar diameter (SEM and LM).

(c) Aperture type (SEM and LM).

(d) Colpus structure (SEM): length of colpus, in equatorial view; width of colpus, in equatorial view; shape of colpus ends, in polar view; surface area of the apocolpium.

(e) Tectum sculpture (SEM): length of spinulae; frequency of spinulae in the mesocolpium; finer tectum sculpture (presence of other structures, except spinulae).

(f) Exine stratification (LM).

Some preparation techniques may influence the size and shape of the pollen grains of representatives of the Asteraceae (Cuzma Velari, 1984) and therefore untreated grains were used. Pollen was obtained from unopened florets, mounted directly onto stubs using a carbon glue ('Leit-C nach Göcke', Neubauer Chemikalien), sputter-coated with gold/palladium and viewed using a Cambridge Stereoscan 250S SEM. Pollen from the same specimens was also prepared for LM studies following the method of Coetzee and Van der Schijff (1979). Although only the shape and size of the pollen grains could be studied from these preparations, the pollen was also cleaned to study colpus structure and tectum sculpture. Initially the pollen grains were cleaned by the acetolysis method of Erdtman (1960). Because of colpar damage and the fact that this method is time-consuming, the filter technique of Bredenkamp and Hamilton-Attwell (1988) was subsequently used.

The polar axis (P), equatorial width (E) and the thickness of the exine and its parts were measured by means of an LM provided with an eye piece micrometer. All measurements were based on a minimum of ten pollen grains per specimen. The frequency of spinulae was determined from an average of three counts in an area of 2 μm × 2 μm (4 μm^2) in the mesocolpium, by using SEM prints of similar magnification.

The descriptive terminology used mainly follows Erdtman (1969) and the attempts at standardization offered by Reitsma (1970) and Nilson and Muller (1978). The shape of pollen grains in equatorial view was determined from the shape classes of Erdtman (1966), while terms proposed by Faegri and Iverson (1975) were used to describe the shape of pollen grains in polar view.

Table 1 Specimens used in the pollen morphological study

Taxon	Collector, number and herbarium
B. discolor subsp. *discolor*	Cilliers 123, 135 (PUC), Hilliard 1673 (UN), Law 5 (NBG), Taylor 206 (UN), Thode A1532 (NH), Venter 1927 (ZULU).
B. discolor subsp. *transvaalensis*	Cilliers 119, 140 (PUC), Gerstner 3013 (NH), Keet 1130 (STE), Schweickerdt 1368 (NH), Strey 8787 (NH).
B. elliptica	Abbott 1349 (NH), Moll 5033 (NH), Pegler 997 (BOL), Schlechter 2652 (NBG), White 1172 (GRA).
B. glabra	Dix 130 (GRA), Fourcade 537 (BOL), Thode A860 (NH), Van Jaarsveld 6881 (NBG), West 267 (BOL).
B. huillensis	Cilliers 104 (PUC), Lang *s.n.* (BOL), Moll & Strey 3755 (NH), Pooley 871 (NH), Ward 3660 (NH).
B. ilicifolia	Bayliss 5052 (GRA), Compton 19654 (NBG), Dahlstrand 1995 (GRA), Feely 62 (NH), Olivier 2445 (GRA).
B. neriifolia	Bolus *s.n.* (NH), Cilliers 75 (PUC), Duthie 540 (BOL), Patterson 1267 (GRA).
B. rotundata	Botha & Ubbink 1556 (PUC), Cilliers 148 (PUC), Galpin 6018 (GRA), Leendertz 235 (GRA), Theron 1557 (PRU).

Results

Polarity, size and symmetry of the pollen grains

The pollen grains of all the taxa are released as monads and are radially symmetrical and isopolar. The polar axis (P) and equatorial width (E) of the pollen grains of *B. huillensis* (P = 30 – 37 μm and E = 26 – 35 μm) are larger than those of all the other species (P = 26 – 34 μm and E = 20 – 30 μm). However, as a result of the overlap that exists in the respective dimensions, it is not possible to distinguish between them on the basis of these characters only. The length of the polar axis of the pollen grains of all the *Brachylaena* taxa studied, is 25 – 50 μm, the grains therefore being medium in size (ME).

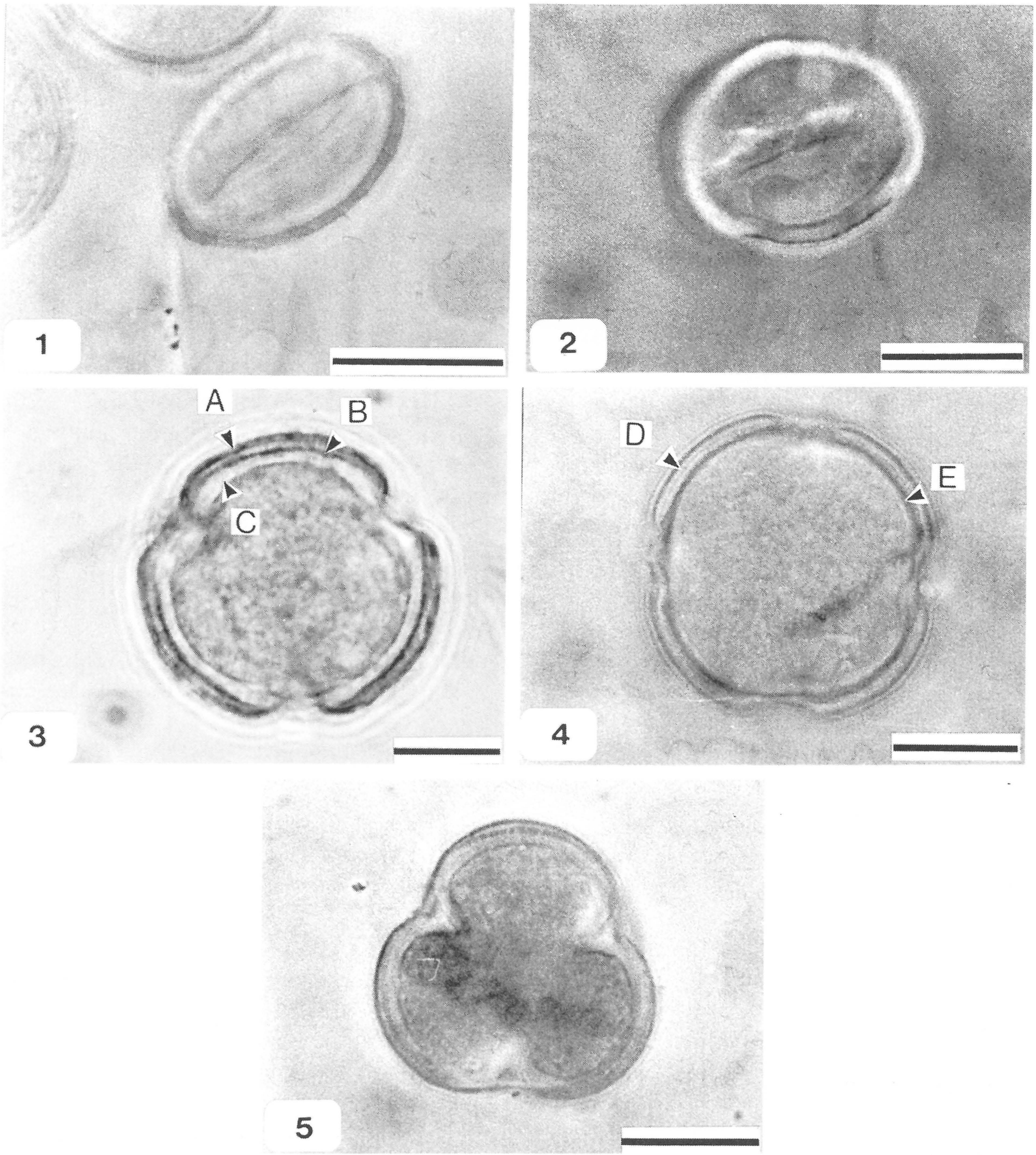
Shape of pollen grains

The shape of the pollen grains of most of the taxa is subprolate (Figure 1), and occasionally prolate spheroidal. Most of the pollen grains of *B. huillensis* are prolate spheroidal (Figure 2), but sometimes oblate spheroidal or subprolate. Since the variation in pollen grain shape is continuous, it is not possible to distinguish *B. huillensis* from other species on the basis of their pollen grain shapes.

The pollen grains of most of the taxa studied appeared spherical in polar view (Figures 3 and 4), and it is therefore not possible to determine whether the grains are angulaperturate or planaperturate. Some of the pollen grains of *B. ilicifolia*, however, appear triangular with somewhat rounded corners (Figure 5) and are clearly planaperturate.

Aperture type

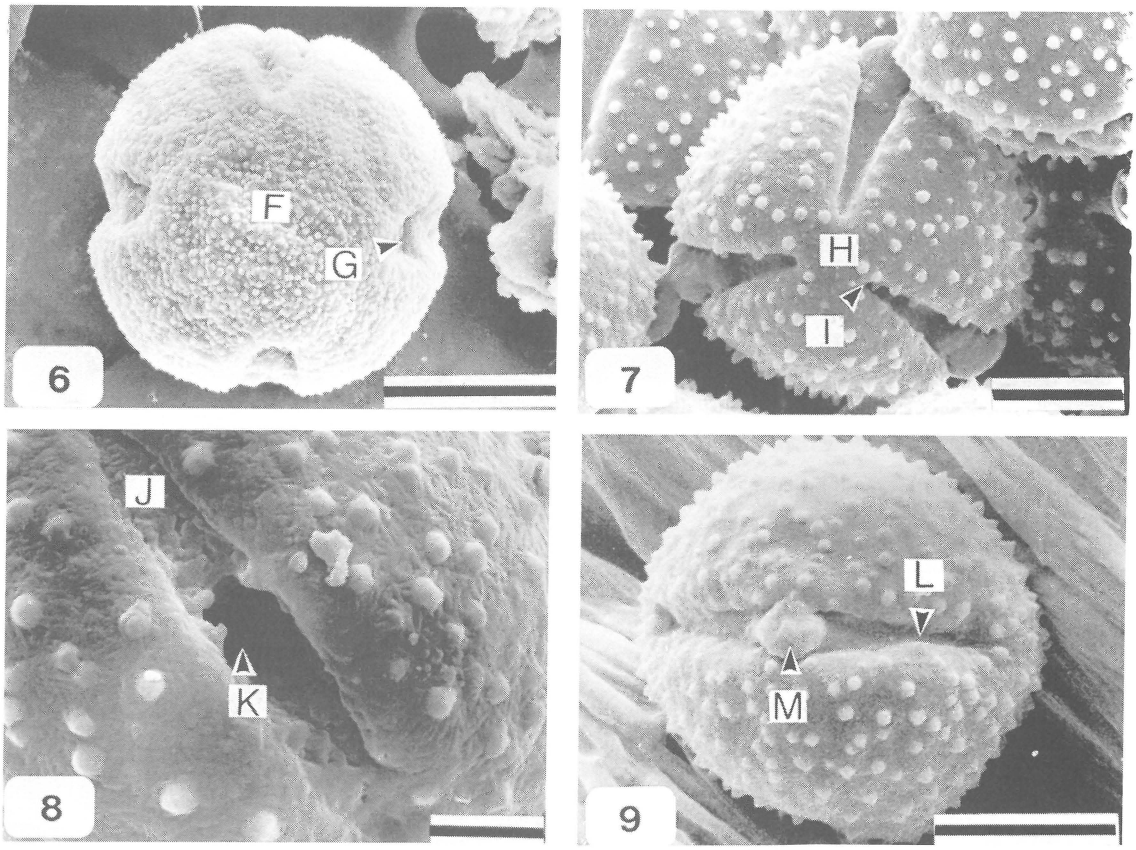
The pollen grains of all the taxa studied are colporate, but the grains differ in the number of their compound apertures (combination of ecto- and endo-apertures). Most of the grains of *B. huillensis* are tetracolporate (rarely tricolporate) (Figure 6). All the other taxa have tricolporate pollen grains (Figure 7). According to NPC analyses all these tricolporate pollen grains have a numerical combination of 345, while the numerical combination of the tetracolporate pollen grains of *B. huillensis* is 445. The ora (endo-aperture) of pollen grains of all the taxa studied is lalongate (Figure 8).



Figures 1 – 5 Light micrographs of pollen grains of *Brachylaena* species. **1.** The equatotial view of an untreated pollen grain of *B. rotundata* (*Theron 1557*). Scale bar = 20 μm. **2.** The equatorial view of an untreated pollen grain of *B. huillensis* (*Cilliers 104*). Scale bar = 20 μm. **3.** A polar optical section through an untreated tricolporate pollen grain of *B. neriifolia* (*Duthie 540*) to show the sexine (A), nexine 1 (B) and nexine 2 (C). Scale bar = 10 μm. **4.** A polar optical section through an untreated tetracolporate pollen grain of *B. huillensis* (*Cilliers 104*) to show the sexine (D) and nexine (E). Scale bar = 10 μm. **5.** A polar optical section through a tricolporate pollen grain of *B. ilicifolia* (*Dahlstrand 1995*). Scale bar = 10 μm.

Colpar structure

On the basis of the size and shape of the colpus (ectoaperture), two groups can be recognized within the taxa studied. Pollen grains of the first group (Figures 7 and 9) possess well-defined colpi that are 18 – 28 μm long and 0.8 – 1 μm wide with pointed ends. Because of the length of the colpi the surface area of the apocolpi is smaller (14 – 27 μm^2) than that of the other group. The surface area of the apocolpi is so small in some pollen grains that these grains appear to be syncolpate. The species belonging to this group are *B. discolor*, *B. elliptica*, *B. glabra*, *B. neriifolia* and *B. rotundata*. The colpi of the pollen grains of representatives of the second group (Figures 6, 10 and 11) are not as well defined and are also shorter (10 – 15 μm) and wider (2 – 2.5 μm) than those of the first group. The ends of these colpi are rounded and the surface area of the apocolpi vary between 100 and 138 μm^2. *B. huillensis* and *B. ilicifolia* are the only representatives of this group.



Figures 6 – 9 Scanning electron micrographs of pollen grains of *Brachylaena* species. **6.** A polar view of an untreated tetracolporate pollen grain of *B. huillensis* (*Cilliers 104*) to show the relative surface area of the apocolpium (F) and the rounded ends of the colpi (G). Scale bar = 10 μm. **7.** A polar view of an untreated tricolporate pollen grain of *B. discolor* subsp. *discolor* (*Cilliers 135*) to show the surface area of the apocolpium (H) and the pointed ends of the colpi (I). Scale bar = 10 μm. **8.** A part of a colpus (J) of a cleaned pollen grain of *B. neriifolia* (*Bolus s.n.*) to show an os (K). Scale bar = 4 μm. **9.** The equatorial view of an untreated pollen grain of *B. elliptica* (*White 1172*) to show the long, relatively thin colpus (L) and the intine (M) pushed through the os (endo-aperture). Scale bar = 10 μm.

Tectum sculpture

Although the tecta of all the taxa studied possess spinulae, two groups can be distinguished on the basis of length and basal width of the spinulae, the number of spinulae per surface area, as well as the finer tectum sculpture (openings present or smooth).

The spinulae of the representatives of the first group (Figure 12) are longer (0.5 – 0.8 μm) and have a wider base (0.5 – 0.9 μm) than those of the second group, with only 2 – 3 spinulae per surface area. Small openings can also be observed on the surface of the tectum. Since no sections were prepared from the pollen grains, it was not possible to determine whether these openings are luminae, or only supratectal in origin. *B. discolor*, *B. elliptica*, *B. glabra*, *B. neriifolia* and *B. rotundata* are the representatives of this group. The tecta of the pollen grains of representatives of the second group (Figure 13) possess shorter spinulae (0.2 – 0.3 μm) with narrower bases (0.2 – 0.4 μm) and can be classified as nanospinulae. They were found to have a considerably larger number of spinulae (20 – 30) per surface area than the first group. Because of this comparatively high spinulae frequency the tectum surface between the spinulae was not clearly visible, but appeared to be smooth. This pollen grain type was found in *B. huillensis* and *B. ilicifolia*.

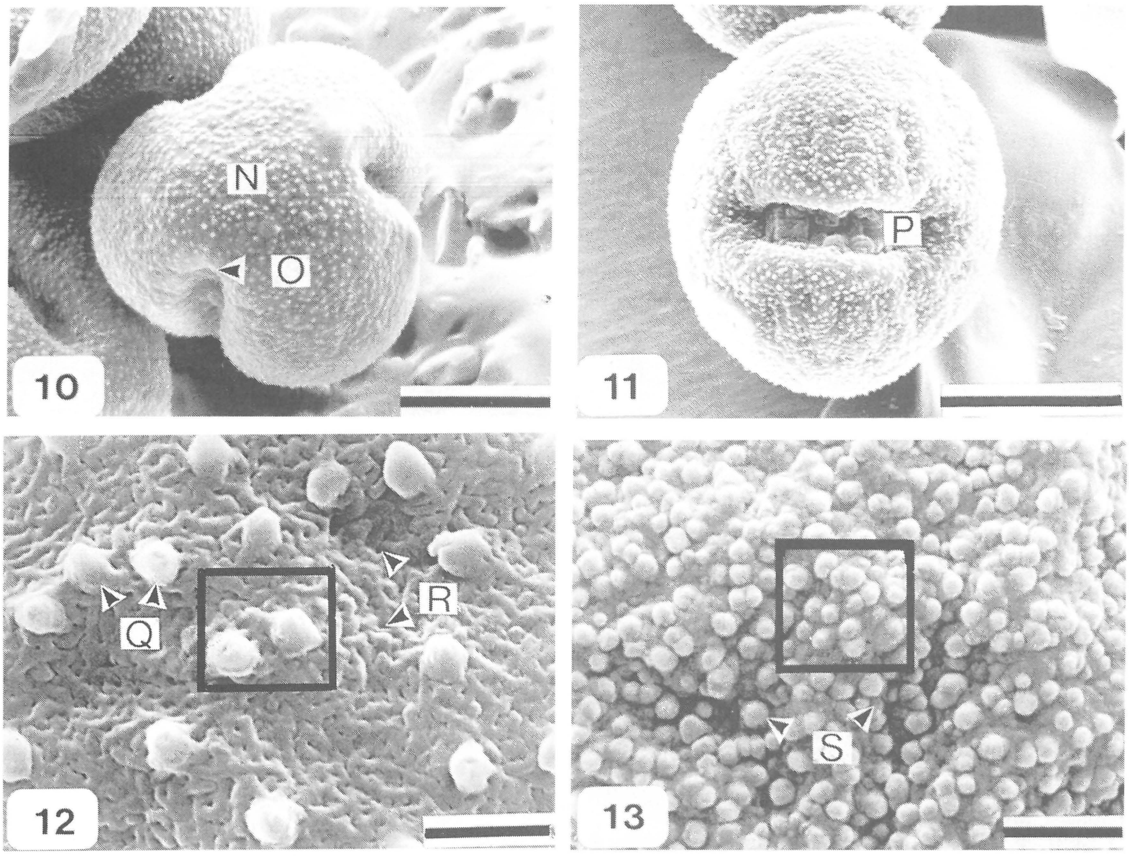
Exine stratification

The exine of most taxa studied consists of a sexine (1 – 2 μm thick) and a nexine (0.8 – 1.2 μm thick) which can be divided into a nexine 1 and a nexine 2 (Figure 3). The nexine part of the exine of *B. huillensis* and *B. ilicifolia* differs from that of the other taxa in that two parts cannot be distinguished and it is also thinner (0.2 – 0.5 μm) (Figures 4 and 5).

Discussion

Additional pollen morphological characters that can be used as taxonomic tools were identified from the SEM studies. These include characters such as the surface area of the apocolpi, spinule frequency and the finer tectum sculpture.

Although pollen morphological characters cannot be used to distinguish amongst all the *Brachylaena* taxa, it is possible to define two groups in the southern African representatives of the genus, on the basis of pollen morphology. *B. discolor* (including *B. uniflora*), *B. elliptica*, *B. glabra*, *B. neriifolia* and *B. rotundata* are included in Group I, while *B. huillensis* and *B. ilicifolia* are the only representatives of Group II. The main pollen morphological distinctions between these two groups are summarized in Table 2. LM observations correspond with most of those of Leins (1971).



Figures 10 – 13 Scanning electron micrographs of pollen grains of *Brachylaena* species. **10.** The polar view of an untreated tricolporate pollen grain of *B. ilicifolia* (*Dahlstrand 1995*) to show the surface area of the apocolpium (N) and the rounded ends of the colpi (O). Scale bar = 10 μm. **11.** The equatorial view of an untreated pollen grain of *B. huillensis* (*Lang s.n.*) to show the short but broad colpus (P). Scale bar = 10 μm. **12.** The finer wall sculpture of a cleaned pollen grain of *B. neriifolia* (*Cilliers 75*) to show the spinulae (Q) and openings on the tectum (R). The square represents that part in which the spinulae were counted. Scale bar = 2 μm. **13.** The finer wall sculpture of a cleaned pollen grain of *B. huillensis* (*Cilliers 104*) to show the nanospinulae (S). The square represents that part in which the nanospinulae were counted. Scale bar = 2 μm.

Characters such as the type of conflorescence, the number of heads per conflorescence and anther length can also be used to distinguish between Groups I and II (Cilliers 1990).

Different aperture types may be found in pollen grains of *B. huillensis* and *B. ilicifolia*. However, they correspond with each other on all the other pollen morphological characters, and are therefore included in one group. No explanation could be found for the variation in aperture type (tricolporate or tetracolporate) in the pollen grains of *B. huillensis*. According to Wodehouse (1935) the number and arrangement of apertures in pollen grains are determined by the arrangement of the grains prior to the formation of a tetrad. He claimed that it is possible for pollen grains with four colpi to develop from four daughter cells which were in a rhombic or even a square arrangement, and not in the usual tetrahedral position.

Most of the southern African taxa of *Brachylaena* (representatives of Group I) possess the *Tarchonanthus camphoratus* pollen type described by Leins (1971). The current study, therefore, shows that these taxa have closer affinities with the genus *Tarchonanthus*. In contrast, *B. huillensis* and *B. ilicifolia* can be clearly differentiated from *Tarchonanthus* on the basis of the colpar structure, tectum sculpture and exine stratification of their pollen grains. It is also evident from this investigation that pollen morphological characters (supported by those reproductive characteristics mentioned earlier) could possibly be used for an infrageneric division of the genus *Brachylaena*. To establish such

Table 2 Summary of the main external morphological distinctions between pollen of Group I (*B. discolor, B. elliptica, B. glabra, B. neriifolia* and *B. rotundata*) and Group II (*B. huillensis* and *B. ilicifolia*) obtained from scanning electron microscope studies

Character	Group I	Group II
Aperture type	tricolporate	tricolporate or tetracolporate
Length of colpus	18 – 28 μm	10 – 15 μm
Width of colpus	0.8 – 1.0 μm	2 – 2.5 μm
Shape of colpus ends	pointed	rounded
Surface area of the apocolpium	14 – 27 μm^2	100 – 138 μm^2
Length of supratectal processes	0.5 – 0.8 μm (spinulae)	0.2 – 0.3 μm (nanospinulae)
Width of spinulae bases	0.5 – 0.9 μm	0.2 – 0.4 μm
Frequency of spinulae (in 4 μm^2)	2 – 3	20 – 30
Finer wall sculpture	openings	smooth (no openings)

a division, pollen morphological information on the *Brachylaena* taxa from Madagascar and the Mascarene Islands will have to be obtained.

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